Protein Purification Test Model

Tuesday, July 05, 2016 3:21 PM

GSR, CH25H and CRYAB

Extract all Proteins using the Clontech Protocol (You should try run a sample test run with BGHT to see if GFP get stuck in the spin column. You find this out by observing the color change between cleared lysate and cleared lysate flow through.

Run Protein Gel (w/ a bit of protein sample) to see if the proteins are correctly eluted and his-tagged.

Measure the concentration of all the protein samples using nanodrop. Record the concentration on the micro-centrifuge tab containing the protein.

Store the protein in -20 ALWAYS and put them in ice when using them for further experiments for **Proof of concept: to package these purified nanoparticles and release these nanoparticles in Fish lens cataracts model to observe any changes in protein aggregation turbidity.**

Expected Band for the Proteins (Note-Histidine tag adds 1kDa to the Protein we extract)

(His-tag not added)

GSR - 56kDa http://www.ptglab.com/Products/GSR-Antibody-18257-1-AP.htm
CH25H - 32KDa
GFP - 27kDa
CRYAA - 20kDa
CRYAB - 20kDa

- You may not necessarily obtain these values. +/- 2~3kb is fine

Harvest Bba_GFP_His_Term Liquid Culture

Saturday, June 11, 2016 9:45 PM

BbaK880005_GFP_His_term Protein Purification

Thursday, June 16, 2016 11:28 AM

Goal: To protein purify GFP from Bba_GFP_His_term

Procedure: xTractor Buffer Sample Preparation & Capturem His-Tagged Purification Miniprep Kit Protocol

Result:

First Tube: LB broth

Second Tube: xTractor Buffer Preparation Sample (contains all the materials from the lysed bacterial cell) (Glows Green possibly because of GFP)

Third Tube: Elution Buffer

Fourth Tube: Eluted His-tagged "GFP" (Glows Green possibly because of GFP)

Conclusion:
Although the Eluted His-tagged solution appeared green under the blue light, we have to run protein gel for the solution to validate that the protein is definitely GFP.

Eluted His-tagged GFP Protein Gel Check

Thursday, June 16, 2016 11:28 AM

Goal: To verify that the eluted protein is GFP from Bba_GFP_His_Term

Procedure: Mini-Protean TGX Precast Gels

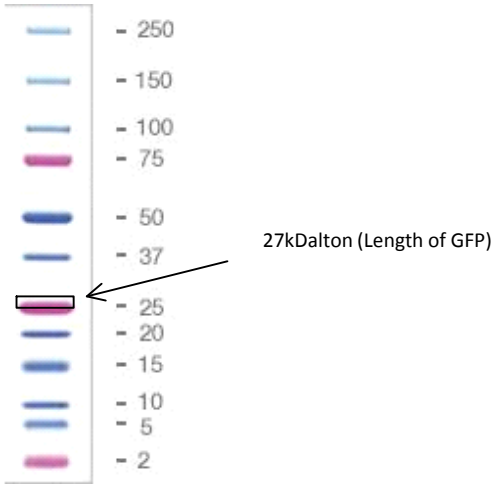
Result:

Lane 1: Protean Dual Color Standard Ladder

Lane 3: xTractor Buffer Preparation Sample (Lysed Bacterial Cell)

Lane 4: Eluted His-Tagged Protein

Conclusion:
No band around 27kDa was seen in Lane 4 which shows that not much GFP could have been in the Eluted His-tagged Protein



Next Step: The amount of GFP in the Eluted solution was too low that we are assuming that no proper band could have been formed. To create more GFP from now we will liquid culture more than 3ml, about 30ml each time.

Harvest Bba_GFP_His_term

Thursday, June 16, 2016 3:58 PM

Goal: To harvest 7ml of Bba_GFP_His_term to yield more pure GFP

Procedure: Same as cloning cycle liquid culture

Result:
Incubated under shaker 180rpm for 16 hours (Standard E.Coli growth period)

Conclusion:

Next Step-> GFP Purification

Bba_K880005_GFP_Histag_Term Protein Purification

2016年6月17日　　下午 04:06

Goal: To protein purify GFP from BBa_GFP_His_Term

Procedure: Protein Purification Protocol Part 1 & Part 2

Used 7ml of liquid culture
Centrifuged the lysed cell and obtained cleared cell lysate for Capturem his-tagged Purification rather than crude cell lysate

Used 300ul for centrifuging down the his-tagged GFP

Result:

Eluted His-Tagged GFP - 0.4 mg/ml

Conclusion:

Concentration is too low. The Eluted His-Tagged GFP barely appeared green under the blue light
Clear cell lysate flow through appeared bright green under the blue light which means that barely any GFP was tagged onto the spin column

Next Step-> We are assuming that there may be no His_term cloned. Waiting for the sequencing result

Bba_K880005_GFP_His_term Sequencing result

Tuesday, October 18, 2016 4:33 PM

There is no Histidine tag in the construct.

Bba_K880005_CH25H_His_Term Protein Purification

2016年9月5日　　下午 02:22

Goal: To protein Purify CH25H from BBaK880005_GSR_Histidine tag _ terminator and BbaK880005 _CH25H (As negative control)

Procedure: (Refer to experiment procedure page) (with a few modifications list below based on the amount of liquid culture)

30ml of liquid culture for both CH25H_His and CH25H

1.2ml of xTractor Buffer was used for both to lyse the cells

5ul of Protease Inhibitor are put into each 1.2ml xTractor buffer-cell solution

Resuspend the pellet in the xTractor buffer

Incubated with gentle shaking 60RPM for 10min at ROOM temperature , 24C

His 60 Ni Resin Column stage after centrifuging 120000g for 20mins in 4c

Conclusion:

No protein was stuck in the column. The concentration of the clear lysate before and after flow through was almost the same. Biuret test was negative for the eluted protein solutions.

Protein Purifying GSR_His and GSR

Friday, September 23, 2016 4:57 PM

Goal: To protein Purify GSR from BBaK880005_GSR_Histidine tag _ terminator and BbaK880005_GSR (As negative control)

Procedure: (Refer to experiment procedure page) (with a few modifications list below based on the amount of liquid culture)

30ml of liquid culture for both GSR_His and GSR

1.2ml of xTractor Buffer was used for both to lyse the cells

5ul of Protease Inhibitor are put into each 1.2ml xTractor buffer-cell solution

Resuspend the pellet in the xTractor buffer

Incubated with gentle shaking 60RPM for 10min at ROOM temperature , 24C

His 60 Ni Resin Column stage after centrifuging 120000g for 20mins in 4c

Conclusion:

No protein was stuck in the column. The concentration of the clear lysate before and after flow through was almost the same.

Protein Purifying CRYAB-His, CRYAB and GFP gene

Tuesday, October 18, 2016 4:34 PM

Goal: To protein Purify CRYAB-His from BBaK880005_CRYAB_Histidine tag _ terminator and BbaK880005 _CRYAB (As negative control). Through protein gel we made sure that CRYAB-His could be expressed. So chose CRYAB - His over GSR-His

Procedure: (Refer to experiment procedure page) (with a few modifications list below based on the amount of liquid culture)

30ml of liquid culture for both CRYAB_His and CRYAB, GFP gene (as negative controls)

1.2ml of xTractor Buffer was used for both to lyse the cells

5ul of Protease Inhibitor are put into each 1.2ml xTractor buffer-cell solution

Resuspend the pellet in the xTractor buffer

Incubated with gentle shaking 60RPM for 10min at ROOM temperature , 24C

His 60 Ni Resin Column stage after centrifuging 120000g for 20mins in 4c

Conclusion:

There was concentration difference between CRYAB His lysate before and after flow through but barely no difference for the negative controls. We assume that Histidine tag in CRYAB-His allowed the protein to be stuck in the column. But somehow we could not elute the protein bound to the column.